

**IN THE
UNITED STATES
PATENT AND TRADEMARK
OFFICE**

<i>Application Number</i>	New - Div. of 09/200,232
<i>Filing Date</i>	Herewith
<i>First Named Inventor</i>	David Mark WHITCOMBE
<i>Group Art Unit</i>	Not Yet Assigned
<i>Examiner Name</i>	Not Yet Assigned
<i>Attorney Docket Number</i>	1991-211

Title of the Invention: **METHODS AND PRIMERS FOR DETECTING TARGET NUCLEIC ACID
SEQUENCES (as amended)**

PRELIMINARY AMENDMENT AND STATEMENT UNDER 37 C.F.R. § 1.821(f)

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

Delete the current title and substitute therefor, --METHODS AND PRIMERS FOR
DETECTING TARGET NUCLEIC ACID SEQUENCES--.

Cancel claims 1-9, and 23. Amend claims 10, 11, 13, 14, 22 and 24 as shown on the
following pages following the amended specification portions.

Delete the fourth full paragraph on page 18, the second full paragraph of page 22, and the
second full paragraph of page 23, of the specification and substitute the replacement
paragraphs contained on the following pages.;

Marked-up copies of the original text of the amended claims, specification portions and
abstract are attached to this amendment. Material inserted is indicated by redlining (**insertion**)
and material deleted is indicated by strike-out (~~strike-out~~).

Clean Copy of Substitute Paragraph, Page 18, Fourth Full Paragraph

Examples

Materials

Primers/Scorpions primers:

B2098-BRCA Scorpions: FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR-HEG-TTGGAGATTTTGTCACTTCCACTCTCAAA (SEQ ID NO: 1)

Underlined regions are the hairpin forming parts, FAM is the fluorescein dye, MR is a non-fluorogenic fluorophore attached to a uracil, HEG is the replication blocking hexethylene glycol monomer. The probe matches the "C-variant" of the BRCA2 polymorphism and mismatches the "A-variant".

R-186-98: untaild equivalent of B2098:TTGGAGATTTTGTCACTTCCACTCTCAAA (SEQ ID NO: 2)

R187-98: opposing primer to the R186-98 and the equivalent Scorpions.

Z3702: the probe segment of the Scorpions B2098:

FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR (SEQ ID NO: 3)

Template DNA: previously genotyped DNA prepared by proteinase K and phenol/chloroform extraction was used at 50ng per 50µl reaction. Genotypes were typically one homozygous A/A, one homozygous C/C and one heterozygote (A/C).

Buffer (1x): 10 mM Tris-HCl (pH 8.3), 1.2 mM or 3.5 mM MgCl₂, 50 mM KCl, dNTPs (each at 100 µM), gelatin at 0.01% (w/v).

Enzyme: AmpliTaq Gold (Perkin-Elmer/ABI) was included in the reaction mix at 2units/50µl reaction.

Clean Copy of Substitute Paragraph, Page 22, Second Full Paragraph

Examples 7 and 8

Random coil embodiment and bimolecular embodiment

Scorpion B2731:

fam-AGGTAGTGCAGAGAGTG-mr-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID NO: 4)

Scorpion B4249 (no quencher on same molecule)

fam-AGGTAGTGCAGAGAGTG-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID NO: 5)

Quencher oligonucleotide (complement of the tail of B4249):

CACTCTCTGCACTACCT-mr (SEQ ID NO: 6)

ARMS primer R284-97: TTCGGGGCTCCACACGGCGACTCTCAAC (SEQ ID NO: 7)

ARMS primer R283-97: TTCGGGGCTCCACACGGCGACTCTCAAG (SEQ ID NO: 8)

Target is the H63D polymorphism of the human hereditary haemochromatosis gene (H/H), B2731 and B4249 are "common" primers to oppose the ARMS primers R283-97, R283-97. Cycling conditions and reaction composition as above. Primers (including *Scorpion* primers) were used at 500nM concentration.

Clean Copy of Substitute Paragraph, Page 23, Second Full Paragraph

Example 9

No quencher embodiment

Scorpion B4249 (no quencher)

fam-AGGTAGTGCAGAGAGTG-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID NO: 5)

ARMS primer R284-97

TTCGGGGCTCCACACGGCGACTCTCAAC (SEQ ID NO: 7)

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